(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 229 814**
B1

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of the patent specification: **31.05.89**

(51) Int. Cl.⁴: **G 01 N 21/05**, G 01 N 15/14

(21) Application number: **86904422.2**

(22) Date of filling: **10.07.86**

(86) International application number: **PCT/NO 86/00050**

(87) International publication number: **WO 87/00282 (15.01.87 Gazette 87/01)**

(54) **A FLOW CHAMBER DEVICE FOR FLOW CYTOMETERS.**

(30) Priority: **10.07.85 NO 852761**

(43) Date of publication of application: **29.07.87 Bulletin 87/31**

(45) Publication of the grant of the patent: **31.05.89 Bulletin 89/22**

(84) Designated Contracting States: **AT CH DE FR LI SE**

(56) References cited:
WO-A-80/02198
DE-A-2 853 703
GB-A-1 574 566

(73) Proprietor: **STEEN, Harald, Wolffsgate 3, N-0358 Oslo 3 (NO)**

(72) Inventor: **STEEN, Harald, Wolffsgate 3, N-0358 Oslo 3 (NO)**

(74) Representative: **Lenz, Franz, AWAPATENT AB Box 5117, S-200 71 Malmö (SE)**

EP 0 229 814 B1

LIBER, STOCKHOLM 1989

Description

The present invention concerns a device which is a flow chamber to be employed in flow cytometers. This device facilitates that the biological cells or other microscopical particles, carried by a microscopical laminar flow of fluid, are brought one by one across the open surface of a plane glass with a velocity which may be varied from above 30 ml sec. to below 1 cm/sec., so that the fluorescence and light scattering of the cells/particles may be measured through optics situated on each side of the plane glass. A further development of the device facilitates that the electrical volume of the cells/particles may be measured simultaneously.

The function of the flow chamber in such instruments is to make particles or biological cells to flow one by one in a highly reproducible manner through the measuring area of the instrument.

The intention of the present invention is to facilitate considerably lower flow velocity and a corresponding increase in the detection sensitivity as compared to other types of flow chambers and furthermore to facilitate the measurement of the electrical volume of the particles/cells simultaneously with meaurement of the photometrical signals.

Flow cytometers are instruments for the measurement of the fluorescence and light scattering properties of microscopical particles. The measurement is carried out when the particles, carried by a laminar flow of fluid of microscopical dimensions, pass one by one through the focus of a beam of excitation light. The pulse of fluorescence emitted by each particle when it passes through this focus is determined by the chemical composition of the particle and is characteristic of this composition. The pulses of fluorescence are detected through appropriate optics by a photoelectric detector which transforms the pulses of fluorescence to equivalent electrical pulses. These pulses are transferred to a multichannel pulse height analyzer (MCA) which meaures the size of the pulses and thereby produces a histogram showing the number of particles as a function of fluorescence intensity.

The light which is scattered by each particle when it passes through the focus of excitation light, and which is determined by the structure and the size of the particle, is detected in a similar manner by another detector and thus gives rise to a histogram which characterizes the particles with regard to structure and size.

The fluid flow in a flow cytometer may have a typical velocity of about 20 m/sec., and the excitation focus which constitutes the measuring area of the instrument may have a size of about 50 µm. This means that each particle is measured in about 3 µsec., which in turn means that it is possible to measure several thousand particles per second. The smallest amount of fluorescent material per particle which may be detected is about $1 \cdot 10^{-16}$ g. Particle sizes below $1 \cdot 10^{-2}$ µm^3 may be measured by light scattering. Both fluorescence and light scattering may be measured with a precision of about 1 %. The most common use of flow cytometers has so far been measurement of the size of biological cells and their content of essential components such as DNA, enzymes and other proteins.

The flow chamber is a critical component in all flow cytometers because the stability of the particle flow with regard to position as well as velocity determines the precision of the measurement. In order to achieve high stability most flow chamber employs hydrodynamic focusing of the particles. Hydrodynamic focusing is achieved when the carrying fluid is forced through the microscopical orifice of a conical nozzle so that a laminar jet of fluid is produced. The particles are introduced in the carrying fluid through a thin cylindrical tube having its axis in common with that of the nozzle and its opening where the nozzle diameter is much larger than the diameter of the cylindrical tube. Thus, the particles will be centered in the microscopical jet which leaves the nozzle.

In one type of flow chambers the free jet produced by the nozzle is used as measuring region in the flow cytometer. In such instruments the excitation light is preferably produced by a laser. In another type of flow chamber the fluid flow is confined to a straight cylindrical or rectangular tube passing through the measuring area. In a third type of flow cytometers, which employs a conventional high pressure arc lamp as the source of excitation light, this tube has a more complicated shape.

It is of critical importance that the flow chamber has optical properties which makes the fluorescence and the light scattering of the flow chamber itself as low as possible in order to achieve the highest possible signal to noise ratio.

Previously, a flow chamber was developed which may be adapted to a fluorescence microscope with incident illumination (epi illumination) through oil immersion optics with high numerical aperture (N.A. = 1.3) which thereby gives optimal intensity of excitation and detection of fluorescence. In this flow chamber the free jet from a nozzle with hydrodynamic focusing falls at an oblique angle on to the open surface of microscope cover glass thereby producing a laminar flow across the surface of the glass, with the particles confined to a narrow sector of this flow (Norwegian patent nr. 144 002). The other side of this cover glass is optically coupled through the oil immersion objective of the fluorescence microscope. The measuring area of this instrument, that is, the focus of the microscope, thus constitutes a microscopical region of the fluid flow adjacent to the surface of the cover glass. The liquid is drained from the cover glass by suction through a thin tube, the opening of which touches the glass surface. The fluid flow across the surface of the cover glass is thus confined between the surface of the cover

glass on the one side and its free surface against the open atmosphere on the other. This configuration has a minimal number of optical surfaces which may scatter light. Furthermore, these surfaces are approximately plane and are situated at right angle to the optical axis of the microscope. This gives this type of flow chamber exceptionally low light scattering and fluorescence and a correspondingly high signal to noise ratio in the measurement of particles. The open configuration of this type of flow chamber has the further advantage that it is very simple to clean.

The amount of fluorescence and scattered light from a particle passing through the measuring area of the flow cytometer is proportional to the time spent by the particle in the measuring area and thus inversely proportional to the velocity of the flow. The sensitivity of the flow cytometer is therefore inversely proportional of the flow cytometer is therefore inversely proportional to the flow velocity. By reducing this velocity it is possible to increase the sensitivity of the instrument correspondingly. In the previous flow chamber (NO-PA 144 002) the nozzle produces a microscopical jet of water in air. In order for the flow chamber to function according to its purpose the laminarity of the jet must be conserved until the jet hits the surface of the cover glass. The condition that such a nozzle shall produce a laminar jet is that the velocity exceeds a certain minimum value given by:

$$v_{min} = 2(2\sigma/d \cdot \rho)^{1/2} \qquad (I)$$

where σ is the surface tension of the fluid, ρ the density of the liquid and d the orifice diameter. This condition limits the sensitivity of all flow cytometers where the cells/particles are carried through the measuring area by a jet in air.

For a nozzle orifice having a diameter of 70 µm, which is a typical value for flow cytometer nozzles, v_{min} = 3 m/sec., while the typical flow velocity is about 10 m/sec. This means that the sensitivity of the instrument can not be increased by more that a factor of 3 by reducing the flow velocity. One major purpose of the present invention is to facilitate a much larger reduction of the flow velocity and thereby a corresponding increase of the detection sensitivity.

Another main purpose is to facilitate measurement of the electrical volume of the particles simutaneously with the measurement of fluorescence and light scattering. A method for measuring the volume of microscopical particles has been known and used for many years. In this method the particles are passed through an opening of microscopical size, e.g. 100 µm diameter, which is filled by an electrolyte, e.g. physological salt solution. On each side of this opening is situated an electrode and between these electrodes, that is, through the opening, a constant electrical current is maintained. The voltage between these electrodes will thus be determined by the electrolytical resistance of the opening. When a particle, which is not electrically conductive, passes through the opening this resistance will increase in proportion to the volume of the particle. The passage of the particle through the opening will thus give rise to a voltage pulse between the electrodes which is prportional to the volume of the particle. Such measurement of the volume of biological cells has proved to be quite useful in combination with the measurement of fluorescence and light scattering in flow cytometers. In the flow chamber mentioned above, as well as other flow systems where the nozzle produces a jet in air, such meaurement is not possible.

The characteristics of the said device are evident primarily from the following claims 1 and 2. Further characteristics of the device according to the invention are evident from the other claims as well as from the following description referring to the included drawings.

Fig. 1 illustrates schematically the device according to the invention.

Fig. 2 illustrates the nozzle which is part of the device according to the invention.

In the present invention is employed a nozzle 1 with hydrodynamic focusing. The nozzle forms a laminar fluid flow across the open surface of a cover glass 2, the other side of which is coupled optically to microscope optics 3. In the present invention, however, the nozzle is formed so that its orifice 4 comes into direct contact with the glass surface 2 (Fig. 1). Hence, the flow from the nozzle does not form a jet in air but rather a fluid meniscus which connects the orifice 4 with the glass surface. In order to give the meniscus a flat form as close to the orifice as possible, which is of critical importance for its optical properties, the nozzle is formed so that its tip has a flat cross section (Fig. 2) the height of which just exceeds the orifice diameter, while the width is several times the height. The nozzle is situated so that one of its plane sides rests against the surface of the cover glass.

Thus, one of the main intentions of the invention is achieved, viz. that the limiting velocity given by equation 1 no longer is a condition for the laminarity of the flow from the nozzle. Experiments show that with this device laminarity can be maintained at least down to velocities of the order of 1 cm/sec., that is a reduction in flow velocity and a corresponding increase in sensitivity, of approximately a factor of 100 as compared to what was possible in our previous flow chamber. The condition for maintaining a stable laminar flow on the surface of the cover glass at these low velocities is that the cover glass 2 is situated vertically so that the fluid runs in the direction of the gravity A tube 5 conducts the fluid away from the cover glass 2

The other main purpose of the invention, that is the meaurement of electrical volume, is achieved by a platinum electrode 6 (thickness 10 µm) which is situated beween the nozzle and the

cover glass surface. Through the meniscus which connects the glass surface with the nozzle orifice this electrode is electrically connected with the other electrode 7 which is situated in the fluid supply 8 of the nozzle 1 (Fig. 1). By maintaining a constant electrolytical current from a current source 10 between the two electrodes it is possible to determine the electrical volume of the particles passing through the orifice by measuring the voltage pulses between the electrodes caused by the particles by means of the voltage meter 11.

The present invention has two other important advantages as compared to our previous flow chamber: Firstly, the low flow velocities make it possible to record images of cells/particles as they pass through the measuring region. The photometrical signals from a cell/particle may thus trigger: 1) a light pulse from an appropriate light source, e.g. a laser, which is focused in the measuring region, and 2) an electronic camera. Secondly, the present invention implies that the distance between the nozzle orifice and the surface of the cover glass is perfectly constant, thus increasing the precision of the path of the particles/cells through the measuring region.

Claims

1. A flow chamber device to be employed in a flow cytometer facilitating that biological cells or particles, carried by a microscopical laminar fluid flow, are brought one by one across the open surface of a plane glass with a velocity which may be varied from above 30 m/s. to below 0.01 m/sec (1 cm/sec.), so that the fluorescence and light scattering of the cells/particles may be measured through optics situated on each side of the plane glass, the flow chamber comprising a nozzle (1) having its axis at an oblique angle to the plane glass (2), characterized in that the nozzle is situated in contact with a open surface of the plane glass so that the orfice (4) of the nozzle is situated immediately adjacent to the glass surface and so that the flow from the orifice of the nozzle connects this orifice with the glass surface through a meniscus of fluid maintained independently of the flow velocity.

2. A flow chamber device to be employed in a flow cytometer facilitating that biological cells or particles, carried by a microscopical laminar fluid flow are brought one by one across the open surface of a plane glass with a velocity which may be varied from above 30 m/sec to below 0.01 m/sec. (1 cm/sec.), so that the fluorescence and light scattering of the cells/particles may be measured through optics situated on each side of the plane glass while at the same time the electrical volume of the cells/particles can be determined, the flow chamber comprising a nozzle (1) having its axis at an oblique angle to the plane glass (2), characterized in that the nozzle is situated in contact with a open surface of the plane glass, so that the orifice (4) of the nozzle is situated immediately adjacent to the glass surface and so that the fluid flow from the nozzle orifice connects the orifice with the glass surface through a meniscus of fluid which is maintained independently of the flow velocity, and that the electrical volume of the cells/particles is determined by means of an electrode (6) of inert metal situated in the plane of contact between said glass and said nozzle, while an electrode (7) is situated in the fluid supply (8) of the nozzle so that a constant electrolytical current between said electrodes is maintained through the nozzle orifice in order to facilitate measurement of the electrical volume of the particles passing through the orifice of said nozzle.

3. A device according to claim 1 or 2, characterized in that the tip of the nozzle (1) has two opposite plane sides so that the cross section of said tip has a height just exceeding the diameter of the orifice (4) of the nozzle and a width which is several times larger than said height.

4. A device according to claim 1 or 2, characterized in that the nozzle (1) rests against the cover glass (2) with one of the plane surfaces of the tip of the nozzle.

5. A device according to one or more of the preceding claims, characterized in that a carrying fluid is introduced in the nozzle (1) so that it runs in a laminar fashion toward the conical orifice (4) while the cells/particles are introduced into the nozzle so that the cells/particles are confined to the central part of the cross section of the flow where it leaves the nozzle orifice (4) and thus confined to a narrow sector of the flow on the surface of the glass (2).

6. A device according to one or more of the preceding claims, characterized in that the glass (2) is situated vertically so that the flow on the glass surface moves in the direction of the gravity.

7. A device according to one or more of the preceding claims, characterized in that a tube (5) is situated at the lower part of said glass (2) thus draining the fluid away from the flow chamber.

8. A device according to one or more of the preceding claims, characterized in that the other side of the glass (2) may be optically coupled to a microscope (3) having Epi-illumination, in such a way that the particle path across the surface of the glass (2) passes through the microscope focus.

Patentansprüche

1. Duchflußvorrichtung für die Verwendung in einem Durchflußzytometer um zu erleichtern, daß in einem mikroskopisch laminaren Flüssigkeisstrom mitgeführe biologische Zellen oder Partikel mit einer zwischen mehr als 30 m/sec und weniger als 0,01 m/sec (1 cm/sec)

variierbaren Geschwindigkeit einzeln über die offene Oberfläche einer Planglasscheibe geführt werden, so daß die Fluoreszenz und Lichtstreuung der Zellen/Partikel mittels an beiden Seiten der Planglasscheibe angeordneter optischer Einrichtungen gemessen werden können, wobei die Durchflußvorrichtung ein Mundstück (1) aufweist, deren Achse in einem geneigten Winkel zur Planglasscheibe ausgerichtet ist, dadurch gekennzeichnet, daß das Mundstück in Berührung mit einer offenen Oberfläche der Planglasscheibe angeordnet ist, so daß die Öffnung (4) des Mundstücks in unmittelbarer Nähe der Glasoberfläche angeordnet ist, und so daß der aus der Öffnung des Mundstücks austretende Flüssigkeitsstrom die Öffnung mit der Glasoberfläche über einen Meniskus der Flüssigkeit verbindet, welcher unabhängig von der Fließgeschwindigkeit erhalten bleibt.

2. Durchflußvorrichtung für die Verwendung in einem Durchflußzytometer um zu erleichtern, daß in einem mikroskopisch laminaren Flüssigkeitsstrom mitgeführte biologische Zellen oder Partikel mit einer zwischen mehr als 30 m/sec und weniger als 0,01 m/sec (1 cm/sec) variierbaren Geschwindigkeit einzeln über die offene Oberfläche einer Planglasscheibe geführt werden, so daß die Fluoreszenz und Lichstreuung der Zellen/Partikel mittels an beiden Seiten der Planglasscheibe angeordneter optischer Einrichtung gemessen werden können und gleichzeitig das elektrische Volumen der Zellen/Partikel bestimmt werden kann, wobei die Durchflußvorrichtung ein Mundstück (1) aufweist, deren Achse in einem geneigten Winkel zur Planglasscheibe ausgerichtet ist, dadurch gekennzeichnet, daß das Mundstück in Berührung mit einer offenen Oberfläche der Planglasscheibe (2) angeordnet ist, so daß die Öffnung (4) des Mundstücks in unmittelbarer Nähe der Glasoberfläche angeordnet ist, und so daß der aus der Öffnung des Mundstücks austretende Flüssigkeitsstrom die Öffnung mit der Glasscheibe über einen Meniskus der Flüssigkeit verbindet, welcher unabhängig von der Fließgeschwindigkeit erhalten bleibt, und daß das elektrische Volumen der Zellen/Partikel mittels einer Elektrode (6) aus inertem Metall ermittelt wird, welche in der Berührungsebene zwischen dem Glas und dem Mundstück angeordnet ist, während eine Elektrode (7) in der Flüssigkeitszufuhr (8) zum Mundstück angeordnet ist, so daß durch die Mundstücksöffnung hindurch ein konstanter elektrolytischer Strom zwischen den Elektroden aufrecht erhalten wird, um die Messung des elektrischen Volumens der. durch die Öffnung des Mundstücks hindurchtretenden Partikel zu ermöglichen.

3. Vorrichtung nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß die Spitze des Mundstücks (1) zwei einander gegenüberliegende ebene Seiten aufweist, so daß der Querschnitt der Spitze eine Höhe hat, welche gerade etwas größer ist als der Durchmesser der Öffnung (4) des Mundstücks, und eine Breite, welche um ein Mehrfaches größer ist als die Höhe.

4. Vorrichtung nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß daß Mundstück (1) mit einer der ebenen Flächen der Spitze des Mundstücks an dem Deckglas (2) anliegt.

5. Vorrichtung nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß die Trägerflüssigkeit derart in das Mundstück (1) eingeführt wird, daß sie in einem laminaren Strom der konischen Öffnung (4) zufließt, während die Zellen/Partikel derart in das Mundstück eingebracht werden, daß sie im mittleren Teil des Querschnitts der Strömung bei deren Austritt aus der Mundstücksöffnung (4) und damit in einem schmalen Bereich der Strömung auf der Oberfläche des Glases (2) zusammengehalten sind.

6. Vorrichtung nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß das Glas (2) senkrecht angeordnet ist, so daß sich die Strömung auf der Glasoberfläche in Richtung der Schwerkraft bewegt.

7. Vorrichtung nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß am unteren Teil des Glases (2) ein Rohr (5) angeordnet ist, über welches die Flüssigkeit von der Vorrichtung abfließen kann.

8. Vorrichtung nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß die andere Seite der Glasscheibe (2) optisch mit einem mit Epi-Illumination versehenen Mikroskop (3) gekoppelt sein kann, derart, daß die Bewegungsbahn der Partikel über die Oberfläche der Glasscheibe (2) durch den Fokuspunkt des Mikroskops hindurch verläuft.

Revendications

1. Appareil à chambre de circulation, destiné à être utilisé dans un cytomètre à circulation facilitant la mise une à une de cellules biologiques ou de particules, transportées par un courant laminaire microscopique de fluide, sur la surface libre d'une lame de verre, avec une vitesse qui peut varier entre plus de 30 m/s et moins de 0,01 m/s (1 cm/s), si bien que la fluorescence des cellules-particules et la diffusion de la lumière par les cellules-particules peuvent être mesurées à l'aide de systèmes optiques disposés de chaque côté de la lame de verre, la chambre de circulation comprenant une buse (1) dont l'axe est incliné en oblique par rapport à la lame de verre (2), caractérisé en ce que la buse est placée au contact d'une surface libre de la lame de verre afin que l'orifice (4) de la buse soit immédiatement adjacent à la surface du verre et que le courant provenant de l'orifice de la buse relie cet orifice à la surface de verre par un ménisque de fluide maintenu

indépendamment de la vitesse du courant.

2. Dispositif à chambre de circulation destiné à être utilisé dans un cytomètre à circulation, facilitant la mise une à une de cellules biologiques ou de particules, transportées par un courant laminaire microscopique de fluide, sur la surface libre d'une lame de verre, avec une vitesse qui peut varier entre plus de 30 m/s et moins de 0,01 m/s (1 cm/s), si bien que la fluorescence des cellules-particules et la diffusion de la lumière par les cellules-particules peuvent être mesurées à l'aide de systèmes optiques placés de chaque côté de la lame de verre, le volume électrique des cellules-particules pouvant être déterminé simultanément, la chambre de circulation comprenant une buse (1) dont l'axe est incliné en oblique par rapport à la lame de verre (2), caractérisé en ce que la buse est placée au contact d'une surface libre de la lame de verre afin que l'orifice (4) de la buse soit immédiatement adjacent à la surface de verre et afin que le courant de fluide provenant de l'orifice de la buse relie l'orifice à la surface du verre par l'intermédiaire d'un ménisque de fluide qui est maintenu indépendamment de la vitesse du courant, et en ce que le volume électrique des cellules-particules est déterminé à l'aide d'une électrode (6) d'un métal inerte placée dans le plan de contact du verre et de la buse, alors qu'une électrode (7) est placée dans la réserve (8) de fluide de la buse si bien qu'un courant électrolytique constant est maintenu entre les électrodes par l'intermédiaire de l'orifice de la buse afin de faciliter la mesure du volume électrique des particules passant par l'orifice de la buse.

3. Appareil selon la revendication 1 ou 2, caractérisé en ce que le bout de la buse (1) a deux côtés plans opposés afin que la section du bout ait une hauteur dépassant juste le diamètre de l'orifice (4) de la buse et une largeur qui est plusieurs fois supérieure à cette hauteur.

4. Appareil selon la revendication 1 ou 2, caractérisé en ce que la buse (1) est en appui contre un couvre-objet (2) par l'une des surfaces planes du bout de la buse.

5. Appareil selon une ou plusieurs des revendications précédentes, caractérisé en ce qu'un fluide de transport est introduit dans la buse (1) afin qu'il s'écoule de manière laminaire vers l'orifice conique (4) alors que les cellules-particules sont introduites dans la buse si bien que les cellules-particules sont confinées dans la partie centrale de la section du courant à l'endroit où celui-ci quitte l'orifice de la buse (4) et sont ainsi confinées à un secteur étroit du courant formé à la surface du verre (2).

6. Appareil selon une ou plusieurs des revendications précédentes, caractérisé en ce que le verre (2) est disposé verticalement afin que le courant formé à la surface du verre se déplace dans la direction de la pesanteur.

7. Appareil selon une ou plusieurs des revendications précédentes, caractérisé en ce qu'un tube (5) est placé dans la partie inférieure du verre (2) et évacue ainsi le fluide de la chambre de circulation.

8. Appareil selon une ou plusieurs des revendications précédentes, caractérisé en ce que l'autre côté du verre (2) peut être couplé optiquement à un microscope (3) ayant un éclairement Epi, de manière que le trajet des particules à la surface du verre (2) passe par le foyer du microscope.

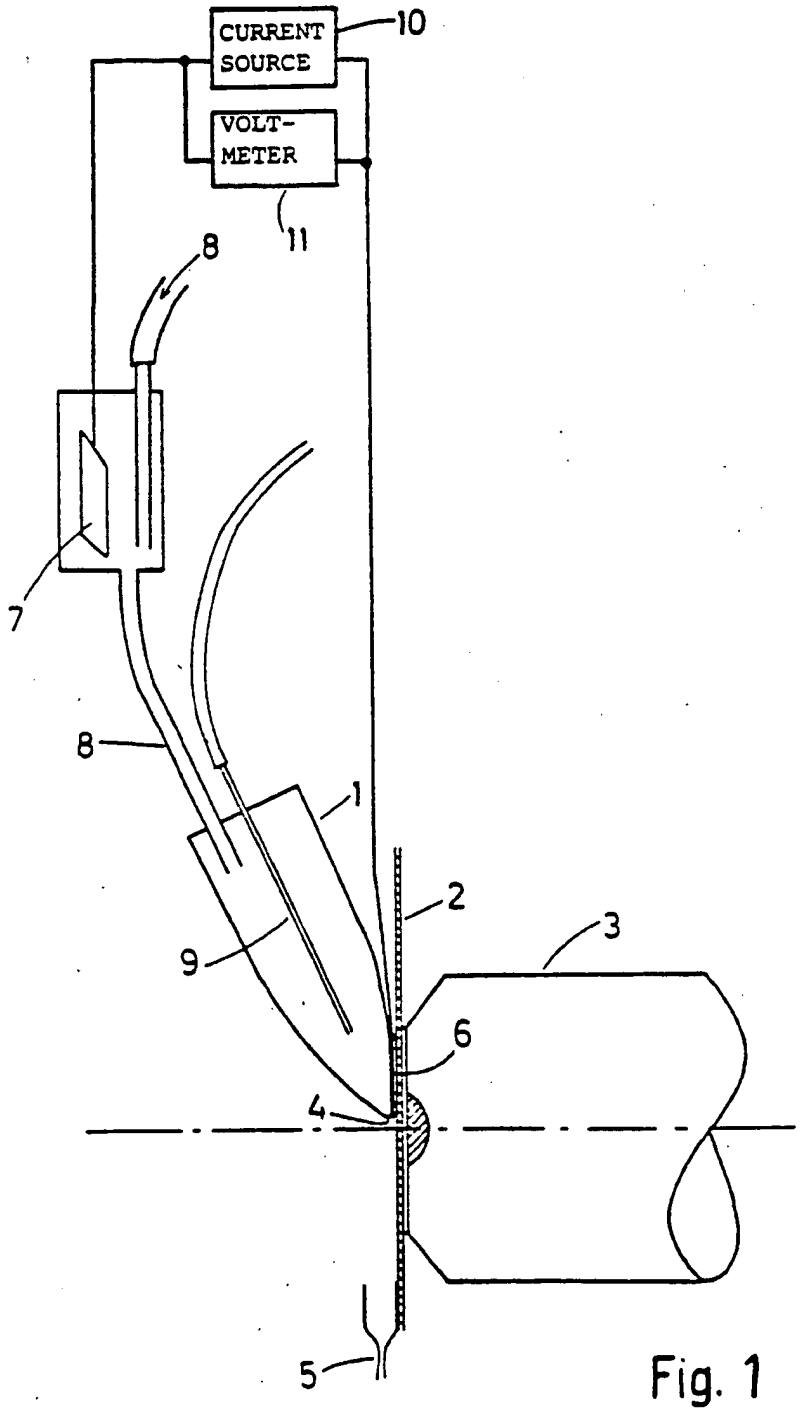

Fig. 1

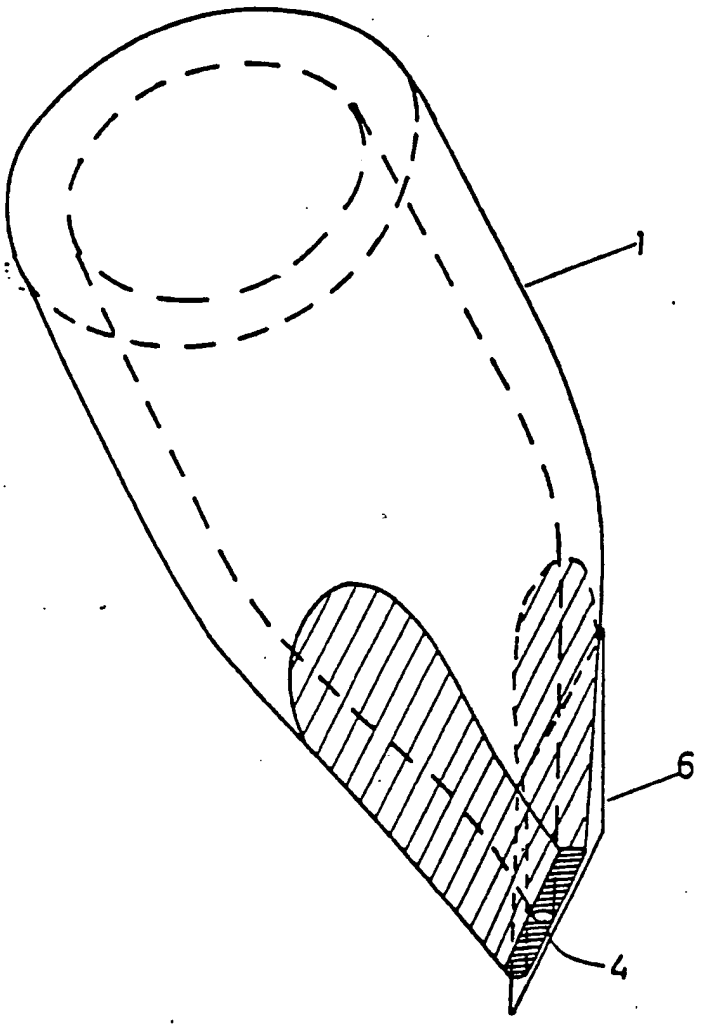

Fig. 2